(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 July 2003 (17.07.2003)

PCT

(10) International Publication Number
WO 03/057204 A2

(51) International Patent Classification[7]: A61K 31/00

(21) International Application Number: PCT/EP03/00079

(22) International Filing Date: 7 January 2003 (07.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2002 00026	8 January 2002 (08.01.2002)	DK
60/346,709	8 January 2002 (08.01.2002)	US

(71) Applicant *(for all designated States except US)*: **NORDIC BIOSCIENCE A/S** [DK/DK]; Herlev Hovedgade 207, DK-2730 Herlev (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CLOOS, Paul, Andreas, Compare** [DK/DK]; Gl. Kalkbraenderivej 62, 5.tv., DK-2100 Kobenhavn 0 (DK).

(74) Agent: **SMART, Peter, J.**; W.H. Beck, Greener & Co., 7 Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/057204 A2

(54) Title: PREVENTION OR ALLEVIATION OF AUTOIMMUNITY

(57) Abstract: Modulation of L-Isoaspartyl (D-Aspartyl) O-Methyltransferase activity in immune system associated cells enables the prevention or alleviation of an autoimmune response by decreasing self-antigen presentation or T-cell proliferation.

PREVENTION OR ALLEVIATION OF AUTOIMMUNITY

The present invention relates to a method for preventing or alleviating an autoimmune response in a mammal by the use of an effective medicament which may act through modulation of L-Isoaspartyl (D-Aspartyl) O-Methyltransferase activity.

Autoimmune diseases are characterized by immune recognition of specific antigens in the patient's own tissue or organs. These antigens are commonly referred to as autoantigens. Depending on the localisation of the target autoantigen and distribution of autoimmune reactions in the organism, autoimmune diseases may be classified as either organ specific or systemic. It is not known why some proteins are prone to become autoantigens. Various possibilities have been suggested: molecular mimicry by bacteria and viruses or release of proteins or peptides from an immune-privileged tissue upon its damage or posttranslational modifications of otherwise tolerated antigens.

The potential role of posttranslational modifications in autoimmunity has been reviewed by Doyle (Doyle and Mamula 2001). One such posttranslational modification is spontaneous isomerisation or racemisation of an amino acid within a protein. Aspartic acid and aspargine will in some proteins undergo this spontaneous reaction in an age dependent fashion, resulting in the formation of L-iso-Asp, D-iso-Asp or D-Asp containing proteins. This reaction has been suggested to play an important role in autoimmunity (Mamula et al 1999; Young et al 2001; patent application WO 01/13110).

Foreign as well as self protein antigens must be broken down within endosomes or lysosomes of the antigen presenting cell (APC), to generate suitable peptides that will form complexes with class II major histo-compatibility complex molecules (MHCII) for presentation to T cells. It has recently been shown that a specific protease called Asparginyl Endo Peptidase (AEP) initially cleaves antigen at asparginyl residues and that antigen is further processed by other proteases such as Cathepsin D and E (Antoniou et al 2000; Hewitt et al 1997; Manoury et al 1998; Manoury 2001). AEP processing appears to be a central event in immune reactions, as AEP-cleavage determines whether certain antigens are presented.

These findings are very important, because they can offer a mechanistic explanation for why isomerisation or racemisation can induce autoimmunity. In the normal situation, AEP cleavage within an aspargine containing antigen fragment will prevent the formation of self-peptides that can form complexes with MHCII for presentation to T cells preventing an immune response to self-protein. If self-proteins are isomerised/ racemised, AEP cleavage is hindered and iso-Asp/ D-Asp containing epitopes within a self-antigen are presented on MHCII leading to T-cell responses and autoimmunity.

The enzyme L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT, PIMT or PCMT), EC 2.1.1.77, is an ubiquitous, mainly cytosolic enzyme which catalyzes transfer of the reactive methyl group of S-adenosyl L-methionine onto the α-carboxyl

group of L-isoaspartyl or D-aspartyl sites in peptides and proteins. Almost every known organism has IAMT or a homologue thereof. IAMT fulfils an important role as repair mechanism for isomerised proteins in the body. IAMT deletion mutants have been shown to possess distinct phenotypes. Mice lacking a functional IAMT gene, exhibit growth retardation and die of fatal seizures at an average age of 42 days (Kim et al 1997). Furthermore these mice have an increased amount of iso-aspartyl containing histone H2B, a possible explanation for the anti-histone antibodies found in systemic lupus erythematosus patients (Young et al 2001).

No studies yet exist on whether the IAMT "repair-system" is altered in any form in autoimmunity. However, amino acid polymorphisms have been identified in human IAMT, which may affect the enzyme's ability to recognise its substrates (David et al 1997; Tsai and Clarke 1994).

IAMT expression levels have been shown to affect apoptosis. An increased IAMT expression level rescues cells from apoptosis, whereas decreased or missing IAMT expression induces elevated levels of apoptosis (Huebscher et al 1999; patent application WO 98/15647). Connections between apoptosis and autoimmunity have been made; in multiple sclerosis a decrease in T-cell apoptosis is observed in the patient group versus healthy individuals (Macchi et al 1999; Zang et al 1999). In other reports an increase in apoptosis has been linked to autoimmunity, where cell death within a tissue provides a supply of putative autoantigens (Rodenburg et al 2000).

In one aspect, the present invention provides a way to regenerate an aspartyl residue to regain cleavage sites for proteases, by increasing IAMT activity in tissue cells (prone for attacks by the immune system) or antigen presenting cells (APC), thereby preventing autoantigen presentation.

This is a very different approach than the apoptotic decrease achieved through increased IAMT activity described in patent application WO 98/15647. According to WO 98/15647 neurodegenerative diseases are associated with increased apoptosis, which implies that neuro-degenerative diseases can be relieved through an increase in IAMT activity as this results in decreased apoptosis. A decrease in apoptosis can also be a disease causing factor, autoimmunity is mentioned briefly as an example of this in WO 98/15647. This would imply that a decrease in IAMT activity (increase in apoptosis) should have a positive effect on autoimmune diseases. Thus increasing IAMT activity in antigen presenting cells (APC) to alleviate autoimmunity as disclosed in the present patent application is the opposite approach to that which would be expected from what was taught in patent application WO 98/15647. Furthermore the self-antigen presenting cells have no direct connection to apoptosis, as the process of presenting an autoantigen will not necessarily lead to cell death.

Not only regulation of IAMT activity in APC can have a positive effect in alleviation or treatment of autoimmune

diseases. It has been shown that T-cells, which lack IAMT hyper-proliferate upon antigen stimulation (Doyle et al 2001). This proliferation is not due to a decrease in apoptosis. Thus, as for the APC, an increase of IAMT activity in T-cells of an autoimmune patient can have a positive effect, by decreasing the immune response to potential autoimmune stimuli.

However, whilst the above mechanisms are currently believed to account for the efficacy of the treatments described herein, the inventive use of the compounds described herein to treat or prevent autoimmune disease is not to be limited by said theories and the invention extends to the effective use of said compounds irrespective of whether they affect IAMT activity.

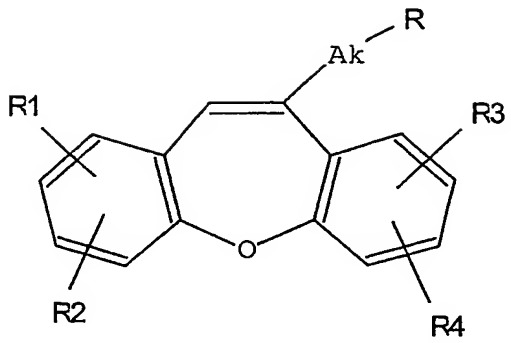
In a first aspect, the present invention provides the use of a regulator of L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT) activity for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/or disease in a mammal.

The invention includes the use of a 10-amino-aliphatyl-dibenz[b,f]oxepine for the preparation of a composition for the prevention, treatment or allevation of an autoimmune response and/or disease in a mammal.

Preferred compounds of this type include 10-amino-

aliphatyl-dibenz[b,f]oxepines of the general formula I:

Formula I



wherein Ak is a divalent aliphatic radical,
R is an amino group that is unsubstituted or mono- or di-substituted by monovalent aliphatic and/or araliphatic radicals or disubstituted by divalent aliphatic radicals, and R1, R2, R3 and R4 are each, independently of the others, hydrogen, lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Compounds of formula I are disclosed in Patent EP 0726265 and US 5780500, hereby incorporated by reference. Further classes of compounds may be envisaged, e.g. compounds involving substitutions, side chain alterations and ring modifications of the above-mentioned oxepines. Such further compounds may be tested applying the method for identifying regulators of IAMT activity, provided in the present invention.

Monovalent aliphatic radicals are, for example, lower alkyl, lower alkenyl or lower alkynyl groups that are unsubstituted or substituted by free or etherified or

esterified hydroxy or by unsubstituted or aliphatically substituted amino, such as lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, lower alkyleneamino-lower alkyl, lower alkenyl, hydroxy-lower alkenyl, lower alkoxy-lower alkenyl, lower alkanoyloxy-lower alkenyl, di-lower alkylamino-lower alkenyl, lower alkynyl, hydroxy-lower alkynyl, lower alkoxy-lower alkynyl, lower alkanoyloxy-lower alkynyl or di-lower alkylamino-lower alkynyl. Araliphatic radicals are, for example, phenyl-lower alkyl radicals that are unsubstituted or substituted by lower alkyl, lower alkoxy, halogen and/or by trifluoromethyl. Amino groups that are mono- or di-substituted by monovalent aliphatic or araliphatic radicals are therefore, for example, lower alkylamino; phenyl-lower alkylamino or phenyl-lower alkyl-lower alkylamino each of which is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen and/or by trifluoromethyl; hydroxy-lower alkylamino, lower alkoxy-lower alkylamino, lower alkanoyloxy-lower alkylamino, lower alkylamino-lower alkylamino, di-lower alkylamino-lower alkylamino, lower alkyleneamino-lower alkylamino, lower alkenylamino, hydroxy-lower alkenylamino, lower alkoxy-lower alkenyl-amino, lower alkanoyloxy-lower alkenylamino, di-lower alkylamino-lower alkenylamino, lower alkynylamino, hydroxy-lower alkynylamino, lower alkoxy-lower alkynylamino, lower alkanoyloxy-lower alkynylamino, di-lower alkylamino-lower alkynylamino, di-lower alkylamino, di(hydroxy-lower alkyl)amino, hydroxy-lower alkyl-lower alkylamino, di(lower alkoxy-lower alkyl)amino, lower alkoxy-lower alkyl-lower alkylamino, lower alkanoyloxy-

lower alkylamino, lower alkanoyloxy-lower alkyl-lower alkylamino, di-lower alkylamino-lower alkylamino, di-lower alkylamino-lower alkyl-lower alkylamino, di-lower alkenylamino, lower alkenyl-lower alkylamino, hydroxy-lower alkenyl-lower alkylamino, di(lower alkoxy-lower alkenyl)amino, lower alkoxy-lower alkenyl-lower alkylamino, lower alkanoyloxy-lower alkenyl-lower alkylamino, di-lower alkylamino-lower alkenyl-lower alkylamino, lower alkynyl-lower alkylamino, lower alkoxy-lower alkynyl-lower alkylamino, lower alkanoyloxy-lower alkynyl-lower alkylamino or di-lower alkylamino-lower alkynyl-lower alkylamino.

Divalent aliphatic radicals are, for example, lower alkylene radicals and, as a component of an amino group disubstituted by a divalent aliphatic radical, also aza-, oxa- or thia-lower alkylene radicals, such as 3- or 4-aza-lower alkylene that is unsubstituted or N-substituted by lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl or by lower alkanoyl, 3- or 4-oxa-lower alkylene or optionally S-oxidised 3- or 4-thia-lower alkylene.

Amino groups disubstituted by divalent aliphatic radicals are, for example, 3- to 8-membered lower alkyleneamino, 3- or 4-aza-lower alkyleneamino that is unsubstituted or N-substituted by lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl or by lower alkanoyl, 3- or 4-oxa-lower alkyleneamino or optionally S-oxidised 3- or 4-thia-lower alkyleneamino, such as, especially, pyrrolidino, piperidino, di-lower alkyl-piperidino, hexamethyleneimino, heptamethyleneimino, piperazino, N'-lower alkylpiperazino,

N'-hydroxy-lower alkylpiperazino, N'-lower alkoxy-lower alkylpiperazino, N'-lower alkanoylpiperazino, morpholino, thiomorpholino, S-oxothiomorpholino or S,S-dioxothiomorpholino.

Hereinbefore and hereinafter there are to be understood by lower radicals and compounds, for example, those having up to and including 7, preferably up to and including 4, carbon atoms.

Lower alkoxy is, for example, C_1-C_7 alkoxy, preferably C_1-C_4 alkoxy, such as methoxy, ethoxy, propyloxy, isopropyloxy, isopropyloxy or butyloxy, but may also be isobutyloxy, sec-butyloxy, tert-butyloxy or a C_5-C_7 alkoxy group, such as a pentyloxy, hexyloxy or heptyloxy group.

Lower alkyl is, for example, C_2-C_7 alkyl, preferably C_1-C_4 alkyl, such as methyl, ethyl, propyl, isopropyl, or butyl or a C_5-C_7 alkyl group, such as a pentyl, hexyl or heptyl group.

Di(hydroxy-lower alkyl)amino is, for example, N,N-di(hydroxy-C_2-C_4 alkyl)amino, such as N,N-di(2-hydroxy-ethyl)amino or N,N-di(3-hydroxypropyl)amino.

Di(lower alkoxy-lower alkenyl)amino is, for example, N,N-di(C_1-C_4 alkoxy-C_2-C_4 alkenyl)amino, such as N,N-di(4-methoxy-but-2-enyl)amino.

Di(lower alkoxy-lower alkyl)amino is, for example, N,N-di(C_1-C_4 alkoxy-C_1-C_4 alkyl)amino, such as N,N-di(2-

methoxyethyl)amino, N,N-di(2-ethoxyethyl)amino or N,N-di(3-methoxypropyl)amino.

Di-lower alkenylamino is, for example, N,N-di-C_2-C_4 alkenylamino, such as N,N-diallylamino or N-methallyl-N-allylamino.

Di-lower alkylamino is, for example, N,N-di-C_1-C_4 alkylamino, such as dimethylamino, diethylamino, ethylmethylamino, dipropylamino, methylpropylamino, ethylpropylamino, dibutylamino or butylmethylamino.

Di-lower alkylamino-lower alkenyl-lower alkylamino is, for example, N-(di-C_1-C_4 alkylamino-C_2-C_4 alkenyl)-N-C_1-C_4 alkylamino, such as N-(4-dimethylaminobut-2-enyl)-N-methylamino.

Di-lower alkylamino-lower alkenylamino is, for example, N-(di-C_1-C_4 alkylamino-C_2-C_4-alkenyl)amino, such as N-(4-dimethylaminobut-2-enyl)amino.

Di-lower alkylamino-lower alkynylamino is, for example, N-(di-C_1-C_4 alkylamino-C_2-C_4-alkynyl)amino, such as N-(4-dimethylaminobut-2-ynyl)amino.

Di-lower alkylamino-lower alkyl-lower alkylamino is, for example, N-(di-C_1-C_4 alkylamino-C_2-C_4 alkyl)-N-C_1-C_4 alkylamino, such as N-(2-dimethylaminoethyl)-N-methylamino, N-(2-dimethylaminoethyl)-N-ethylamino, N-(3-dimethylaminopropyl)-N-methylamino or N-(4-dimethylaminobutyl)-N-methylamino.

Di-lower alkylamino-lower alkylamino is, for example, N-(di-C_1-C_4 alkylamino-C_2-C_4 alkyl)amino, such as N-(2-dimethylaminoethyl)amino, N-(2-dimethylaminoethyl) amino, N-(3-dimethylaminopropyl)amino or N-(4-dimethyl-aminobutyl)amino.

Halogen is, for example, halogen having an atomic number of up to and including 35, such as chlorine or bromine.

Hydroxy-lower alkenyl-lower alkylamino is, for example, N-(hydroxy-C_2-C_4 alkenyl)-N-(C_1-C_4 alkylamino, such as N-(4-hydroxybut-2-enyl)-N-methylamino.

Hydroxy-lower alkenylamino is, for example, hydroxy-C_2-C_4 alkenylamino, such as 4-hydroxybut-2-enylamino.

Hydroxy-lower alkynylamino is, for example, hydroxy-C_2-C_4 alkynylamino, such as 4-hydroxybut-2-ynylamino.

Hydroxy-lower alkyl-lower alkylamino is, for example, N-(hydroxy-C_2-C_4 alkyl)-N-C_1-C_4 alkyl-amino, such as N-(2-hydroxyethyl)-N-methylamino, N-(3-hydroxy-propyl)-N-methylamino or N-(4-hydroxybutyl)-N-methyl-amino.

Hydroxy-lower alkylamino is, for example, hydroxy-C_2 -C_4 alkylamino, such as 2-hydroxyethylamino, 3-hydroxy-propylamino or 4-hydroxybutylamino.

N'-Hydroxy-lower alkylpiperazino is, for example, N'-(hydroxy-C_1-C_4 alkyl)piperazino, such as N'-(2-hydroxyethyl)piperazino or N'-(3-hydroxypropyl) piperazino.

N'-Lower alkanoylpiperazino is, for example, N'-C_1-C_7 alkanoylpiperazino, such as N'-acetylpiperazino.

N'-Lower alkoxy-lower alkylpiperazino is, for example, N'-(C_1-C_4 alkoxy-C_1-C_4 alkyl)piperazino, such as N'-(2-methoxyethyl)piperazino or N'-(3-methoxypropyl) piperazino.

N'-Lower alkylpiperazino is, for example, N'-C_1-C_4 alkylpiperazino, such as N'-methylpiperazino, N'-ethylpiperazino, N'-propylpiperazino or N'-butyl-piperazino.

Lower alkoxy is, for example, C_1-C_7 alkoxy, preferably C_1-C_7 alkoxy, such as methoxy, ethoxy, propyloxy, isopropyloxy, butyloxy, isobutyloxy, sec-butyloxy, tert-butyloxy, pentyloxy or a hexyloxy or heptyloxy group.

Lower alkanoyloxy-lower alkenyl-lower alkylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4 alkenyl)-N-(C_1-C_4 alkyl)amino, such as N-(4-acetoxybut-2-enyl)-N-methyl-amino.

Lower alkanoyloxy-lower alkenylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4-alkenyl)amino, such as N-(4-acetoxybut-2-enyl)amino.

Lower alkanoyloxy-lower alkynyl-lower alkylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4 alkynyl)-N-(C_1-C_4 alkyl)amino, such as N-(4-acetoxybut-2-ynyl)-N-methyl-amino.

Lower alkanoyloxy-lower alkynylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4-alkynyl)amino, such as N-(4-acetoxybut-2-ynyl)amino.

Lower alkanoyloxy-lower alkyl-lower alkylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4 -alkyl)-N-(C_1-C_4 lkyl)amino, such as N-(2-acetoxyethyl)-N-methylamino, N-(2-acetoxyethyl)-N-ethylamino, N-(3-acetoxypropyl)-N-methylamino or N-(4-acetoxybutyl)-N-methylamino.

Lower alkanoyloxy-lower alkylamino is, for example, N-(C_1-C_7 alkanoyloxy-C_2-C_4 alkyl)amino, such as N-(2-acetoxyethyl)amino, N-(3-acetoxypropyl)amino or N-(4-acetoxybutyl)amino.

Lower alkenyl-lower alkylamino is, for example, N-(C_2-C_7 alkenyl)-N-(C_2-C_7 alkyl)amino, especially N-(C_2-C_4 alkenyl)-N-(C_1-C_4 alkyl)amino, such as N-vinyl-N-methylamino, N-allyl-N-methylamino, N-allyl-N-ethylamino, N-but-2-enyl-N-methylamino or N-but-3-enyl-N-methyl amino. Lower alkenylamino is, for example, N-(C_2-C_7 alkenyl)amino, especially N-(C_2-C_4-alkenyl)amino, amino, such as vinylamino, allylamino, but-2-enylamino or N-but-3-enylamino, especially allylamino.

Lower alkynyl-lower alkylamino is, for example, N-(C_2-C_4 alkynyl)-N-(C_1-C_4 alkyl)amino, such as N-propargyl-N-methylamino, N-but-2-ynyl-N-methylamino or N-but-3-ynyl-N-methylamino.

Lower alkynylamino is, for example, N-(C2 -C7 alkynyl)amino, especially N-(C_2-C_4 alkynyl)amino, such as propargylamino, but-2-ynylamino or N-but-3-ynylamino, especially propargylamino.

Lower alkoxy is, for example, C_1-C_7 alkoxy, preferably C_1-C_4 alkoxy, such as methoxy, ethoxy, propyloxy, isopropyloxy or butyloxy, but may also be isobutyloxy, sec-butyloxy, tert-butyloxy or a C_5-C_7 alkoxy group, such as a pentyloxy, hexyloxy or heptyloxy group.

Lower alkoxy-lower alkenyl-lower alkylamino is, for example, N-(C_1-C_4 alkoxy-C_2-C_4 alkenyl)-N-(C_1-C_4 alkyl) amino, such as N-(4-methoxybut-2-enyl)-N-methyl-amino, N-(4-methoxybut-2-enyl)-N-ethylamino or N-(4-ethoxybut-2-enyl)-N-methylamino.

Lower alkoxy-lower alkenylamino is, for example, N-(C_1-C_4 alkoxy-C_2-C_4 alkenyl)amino, such as N-(4-methoxybut-2-enyl)amino or N-(4-ethoxybut-2-enyl)amino.

Lower alkoxy-lower alkynyl-lower alkylamino is, for example, N-(C_1-C_4 alkoxy-C_2-C_4 alkynyl)-N-(C_1-C_4 alkyl) amino, such as N-(4-methoxybut-2-ynyl)-N-methylamino, N-(4-methoxybut-2-ynyl)-N-ethylamino or N-(4-ethoxybut-2-ynyl)-N-methylamino.

Lower alkoxy-lower alkynylamino is, for example, N-(C_1-C_4 alkoxy-C_2-C_4 alkynyl)amino, such as N-(4-methoxybut-2-ynyl)amino, N-(4-ethoxybut-2-ynyl)amino or N-(4-propyloxybut-2-ynyl)amino.

Lower alkoxy-lower alkylamino is, for example, C_1-C_4 alkoxy-C_2-C_4 alkylamino, such as 2-methoxyethylamino, 2-ethoxyethylamino, 2-propyloxyethylamino, 3-methoxypropylamino, 3-ethoxypropylamino, 4-methoxybutylamino, 2-isopropyloxyethylamino or 2-butyloxyethylamino.

Lower alkoxy-lower alkyl-lower alkylamino is, for example, N-(C_1-C_4 alkoxy-C_2-C_4 alkyl)-N-(C_1-C_4 alkyl)amino, such as N-(2-methoxyethyl)-N-methylamino, N-(2-ethoxy-ethyl)-N-methylamino, N-(2-propyloxyethyl)-N-methylamino, N-(3-methoxypropyl)-N-methylamino, 3-ethoxypropylamino or N-(4-methoxybutyl)-N-methylamino.

Lower alkyl is, for example, C_1-C_7 alkyl, preferably C_1-C_4 alkyl, such as methyl, ethyl, propyl, isopropyl or butyl, but may also be isobutyl, sec-butyl, tert-butyl or a C_5-C_7 alkyl group, such as a pentyl, hexyl or heptyl group.

Lower alkylamino is, for example, C_1-C_7 alkylamino, preferably C_1-C_4 alkylamino, such as methylamino, ethylamino, propylamino, isopropylamino or butylamino, but may also be isobutylamino, sec-butylamino or tert-butylamino or a C5 -C7 alkylamino group, such as a pentylamino, hexylamino or heptylamino group, and is especially methylamino or propylamino.

Lower alkylamino-lower alkylamino is, for example, N-(C_1-C_4 alkylamino-C_2-C_4)amino, such as N-(2-methyl-aminoethyl)amino, N-(3-methylaminopropyl)amino, N-(4-

methylaminobutyl)amino, N-(2-ethylaminoethyl)amino, N-(3-ethylaminopropyl)amino or N-(4-ethylaminobutyl)amino.

Lower alkyleneamino-lower alkylamino is, for example, 3- to 8-membered alkyleneamino-C_2-C_4 alkylamino, such as 2-pyrrolidinoethylamino, 2-piperidinoethylamino, 2-dimethylpiperidinoethylamino, 2-hexamethyleneimino-ethylamino, 3-pyrrolidinopropylamino, 3-piperidino-propylamino, 3-dimethylpiperidinopropylamino or 3-hexamethyl-eneiminopropylamino.

Phenyl-lower alkyl-lower alkylamino is, for example, N-(phenyl-C_1-C_4 alkyl)-N-(C_1-C_4 alkyl)amino, such as N-benzyl-N-methylamino, N-(2-phenylethyl)-N-methylamino or N-(4-phenylbutyl)-N-methylamino.

Phenyl-lower alkylamino is, for example, phenyl-C_1-C_4 alkylamino, such as benzylamino, 1- or 2-phenyl-ethylamino, 3-phenylpropylamino or 4-phenylbutylamino.

Salts of compounds of formula I are, for example, the pharmaceutically acceptable acid addition salts thereof with suitable mineral acids, such as hydrohalic acids, sulfuric acid or phosphoric acid, for example hydrochlorides, hydrobromides, sulfates, hydrogen sulfates or phosphates, or salts with suitable aliphatic or aromatic sulfonic acids or N-substituted sulfamic acids, for example methanesulfonates, benzenesulfonates, p-toluenesulfonates or N-cyclohexylsulfamates (cyclamates).

Preferably, ak is lower alkylene,

R is amino, lower alkylamino; phenyl-lower alkylamino or phenyl-lower alkyl-lower alkylamino each of which is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen and/or by trifluoromethyl; hydroxy-lower alkylamino, lower alkoxy-lower alkylamino, lower alkanoyloxy-lower alkylamino, lower alkylamino-lower alkylamino, di-lower alkylamino-lower alkylamino, lower alkyleneamino-lower alkylamino, lower alkenylamino, hydroxy-lower alkenylamino, lower alkoxy-lower alkenylamino, lower alkanoyloxy-lower alkenylamino, di-lower alkylamino-lower alkenylamino, lower alkynylamino, hydroxy-lower alkynylamino, lower alkoxy-lower alkynylamino, lower alkanoyloxy-lower alkynylamino, di-lower alkylamino-lower alkynylamino, di-lower alkylamino, di(hydroxy-lower alkyl)amino, hydroxy-lower alkyl-lower alkylamino, di(lower alkoxy-lower alkyl)amino, lower alkoxy-lower alkyl-lower alkylamino, lower alkanoyloxy-lower alkylamino, lower alkanoyloxy-lower alkyl-lower alkylamino, di-lower alkylamino-lower alkylamino, di-lower alkylamino-lower alkyl-lower alkylamino, di-lower alkenylamino, lower alkenyl-lower alkylamino, hydroxy-lower alkenyl-lower alkylamino, di(lower alkoxy-lower alkenyl)amino, lower alkoxy-lower alkenyl-lower alkylamino, lower alkanoyloxy-lower alkenyl-lower alkylamino, di-lower alkylamino-lower alkenyl-lower alkylamino, lower alkynyl-lower alkylamino, lower alkoxy-lower alkynyl-lower alkylamino, lower alkanoyloxy-lower alkynyl-lower alkylamino, di-lower alkylamino-lower alkynyl-lower alkylamino, 3- to 8-membered lower alkyleneamino; 3- or 4-aza-lower alkyleneamino that is unsubstituted or N-substituted by lower alkyl, hydroxy-lower alkyl, lower

alkoxy-lower alkyl or by lower alkanoyl; 3- or 4-oxa-lower alkyleneamino or optionally S-oxidised 3- or 4-thia-lower alkyleneamino andR1, R2, R3 and R4 are each, independently of the others, hydrogen, lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Alternatively, ak is lower alkylene,
R is amino, lower alkylamino; phenyl-lower alkylamino or phenyl-lower alkyl-lower alkylamino each of which is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen and/or by trifluoromethyl; lower alkenylamino, lower alkynylamino, di-lower alkylamino, 3- to 8-membered lower alkyleneamino; 3- or 4-aza-lower alkyleneamino that is unsubstituted or N-substituted by lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl or by lower alkanoyl; 3- or 4-oxa-lower alkylene-amino or optionally S-oxidised 3- or 4-thia-lower alkyleneamino and
R1, R2, R3 and R4 are each, independently of the others, hydrogen, lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Most preferably, ak is methylene,
R is amino, C1 -C4 alkylamino, such as methylamino, ethylamino, propylamino or butylamino; phenyl-C1 -C4 alkylamino, such as benzylamino or phenethylamino, that is unsubstituted or substituted by C1 -C4 alkyl, such as methyl, C1 -C4 alkoxy, such as methoxy, halogen having an atomic number of up to and including 35, such as chlorine or bromine, and/or by trifluoromethyl; phenyl-C1 -C4 alkyl-C1 -C4 alkylamino, such as N-benzyl-N-methylamino, that is unsubstituted or substituted by C1 -C4 alkyl, such as

methyl, C1 -C4 alkoxy, such as methoxy, halogen having an atomic number of up to and including 35, such as chlorine or bromine, and/or by trifluoromethyl; C2 -C7 alkenylamino, such as allylamino, methallylamino or but-2-enylamino, C2 -C7 alkynylamino, such as propargylamino or but-2-ynylamino, N-C2 -C7 alkenyl-N-C1 -C4 alkylamino, such as N-allyl-N-methylamino, N-allyl-N-ethylamino, N-methallyl-N-methylamino or N-but-2-enyl-N-methylamino, N-C2 -C7 alkynyl-N-C1 -C4 alkylamino, such as N-propargyl-N-methylamino, N-propargyl-N-ethylamino or N-but-2-ynyl-N-methylamino, di-C1 -C4 alkylamino, such as dimethyl-amino, diethylamino, N-methyl-N-propylamino or N-butyl-N-methylamino, pyrrolidino, piperidino, morpholino, piperazino, N'-C1 -C4 alkylpiperazino, such as N'-methylpiperazino, or N'-(hydroxy-C2 -C4 alkyl)piperazino, such as N'-(2-hydroxyethyl)piperazino, and

R1, R2, R3 and R4 are each, independently of the others, hydrogen, C1 -C4 alkyl, such as methyl, C1 -C4 alkoxy, such as methoxy, halogen having an atomic number of up to and including 35, such as chlorine or bromine, or trifluoromethyl, or

R is C2 -C7 alkenylamino, such as allylamino, methallylamino or but-2-enylamino, C2 -C7 alkynylamino, such as propargylamino or but-2-ynylamino, N-C2 -C7 alkenyl-N-C1 -C4 alkylamino, such as N-allyl-N-methyl-amino, N-allyl-N-ethylamino, N-methallyl-N-methylamino or N-but-2-enyl-N-methylamino, N-C2 -C7 alkynyl-N-C1 -C4 alkylamino, such as N-propargyl-N-methylamino, N-propargyl-N-ethylamino or N-but-2-ynyl-N-methylamino, or

pPyrrolidino, piperidino or morpholino,

R1 and R3 are each, independently of the others, hydrogen, C1 -C4 alkyl, such as methyl, C1 -C4 alkoxy, such as methoxy, halogen having an atomic number of up to and including 35, such as chlorine or bromine, or trifluoromethyl, and
R2 and R4 are hydrogen, or
R is C2 -C7 alkenylamino, such as allylamino, methallylamino or but-2-enylamino, C2 -C7 alkynylamino, such as propargylamino or but-2-ynylamino, N-C2 -C7 alkenyl-N-C1 -C4 alkylamino, such as N-allyl-N-methylamino, N-allyl-N-ethylamino, N-methallyl-N-methyl-amino or N-but-2-enyl-N-methylamino, N-C2 -C7 alkynyl-N-C1 -C4 alkylamino, such as N-propargyl-N-methylamino, N-propargyl-N-ethylamino or N-but-2-ynyl-N-methylamino, or phenyl-C1 -C4 alkylamino, such as benzylamino or phenethylamino, that is unsubstituted or substituted by C1 -C4 alkyl, such as methyl, C1 -C4 alkoxy, such as methoxy, halogen having an atomic number of up to and including 35, such as chlorine or bromine, and/or by trifluoromethyl, and
R1, R2, R3 and R4 are hydrogen,

The invention relates specifically to the use of
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
N-allyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-allyl-N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-prop-2-ynylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-propylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-propylamine;
1-dibenz[b,f]oxepin-10-ylmethyl-piperidine;

4-dibenz[b,f]oxepin-10-ylmethyl-morpholine;
N-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop2-ynylamine;
1-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-propylamine;
N-methyl-N-prop-2-ynyl-N-(3-trifluoromethyl-dibenz [b,f]oxepin-10-ylmethyl)amine 1-(3-trifluoromethyl dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(7-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(7-chloro-dibenz[b,f]oxepin- 10-ylmethyl)-pyrro-lidine;
N-(8-methoxy-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
N-(8-tert-butyl-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(8-tert-butyl-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(6-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(6-chloro-dibenz[b,f]oxepin-10-ylmethyl) pyrrolidine;
N-(1-fluoro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(1-fluoro-dibenz[b,f]oxepin-10-ylmethyl) pyrrolidine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-benzylamine;
N-benzyl-N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-propyl-N-benzylamine;
N-allyl-N-benzyl-N-(dibenz[b,f]oxepin-10-ylmethyl) amine;
1-(dibenz[b,f]oxepin-10-ylmethyl)-4-methyl-piperazine;
1-(dibenz[b,f]oxepin-10-ylmethyl)-4-(2-hydroxy-ethyl)-piperazine;
N, N-diethyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine;

N-(dibenz[b,f]oxepin-10-ylmethyl)-N,N-dimethylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine;
1-(dibenz[b,f]oxepin-10-ylmethyl)pyrrolidine;
N-[1-(dibenz(b,f]oxepin-10-ylethyl)-N,N-dimethyl-amine;
N-(1-(dibenz[b,f]oxepin- 10-ylethyl)-N-methylamine;
1-(8-methoxy-dibenz[b,f]oxepin-10-ylmethyl)-4-methylpiperazine;
N-(8-methoxy-dibenz[b,f]oxepin- 10-ylmethyl)-N,N-dimethylamine;
N-(8-methoxy-dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine:
N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-butyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-(8-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N,N-dimethylamine and
N-(8-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N,N-diethylamine and of pharmaceutically acceptable salts thereof, and also to N-(dibenz[b,f]oxepin- 10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
N-allyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-allyl-N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-prop-2-ynylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-propylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-propylamine;
1-dibenz[b,f]oxepin-10-ylmethyl-piperidine;
4-dibenz[b,f]oxepin-10-ylmethyl-morpholine;
N-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-propylamine;

N-methyl-N-prop-2-ynyl-N-(3-trifluoromethyl-dibenz[b,f]oxepin-10-ylmethyl)amine;
1-(3-trifluoromethyl-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(7-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(7-chloro-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(8-methoxy-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
N-(8-tert-butyl-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(8-tert-butyl-dibenz[b,f]oxepin-10-ylmethyl)-pyrrolidine;
N-(6-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(6-chloro-dibenz[b,f]oxepin-10-ylmethyl) pyrrolidine;
N-(1-fluoro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine;
1-(1-fluoro-dibenz[b,f]oxepin-10-ylmethyl) pyrrolidine;
N-benzyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine;
N-benzyl-N-(dibenz[b,f]oxepin-10-ylmethyl)-N-methylamine;
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-propyl-N-benzylamineand
N-allyl-N-benzyl-N-(dibenz[b,f]oxepin-10-ylmethyl)amine and to the use of pharmaceutical compositions comprising them.

According to one preferred practice of the invention, wherein ak is methylene, R is amino; phenyl-C_1-C_4 alkylamino unsubstituted or substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen having an atomic number of up to and including 35 and/or by trifluoromethyl; N-phenyl- C_1-C_4 alkyl-N- C_1-C_4 alkylamino unsubstituted or substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen having an atomic number of up to and

including 35 and/or by trifluoromethyl; or C_2-C_7 alkenylamino, C_2-C_7 alkynylamino, N- C_2-C_7 alkenyl-N- C_1-C_4-alkylamino or N- C_1-C_4 alkynyl-N- C_1-C_4 alkylamino, and R_1, R_2, R_3 and R_4 are each, independently of the others, hydrogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen having an atomic number of up to and including 35 or trifluoromethyl, or a salt thereof.

Preferably, effect of the compound to be administered is an up-regulation of IAMT activity.

The invention includes a method for diagnosis or risk assessment in relation to autoimmunity comprising either screening for genetic polymorphisms in the IAMT gene or quantification of IAMT gene transcription level, protein level or activity, in a sample.

The IAMT protein or derivatives thereof, preferably in a suitable pharmaceutical composition, can according to the present invention be used to prevent, treat or alleviate an autoimmune response or disease.

Another way to modulate IAMT activity and thereby influence an autoimmune response according to the invention, is to provide a IAMT encoding nucleic acid sequence or a functional derivative thereof to a patient. Especially a pharmaceutical composition including an expression vector with the IAMT gene regulated by a specific promoter is presented in the present invention.

The fundamental aspect of the present invention is the ability to influence an autoimmune response, preferably

preventing, treating or alleviating it, through the regulation of IAMT activity. Preference is given to mammalian IAMT, in particular human, canine,feline and rodent IAMT (Swiss Prot accession nr. Human P22061, Mouse P23506, Rat P22062, Dog and Cat are still unresolved).

As used herein, "antibody" means polyclonal, monoclonal or humanized antibodies, including Fc fragments, Fab fragments, chimeric antibodies or other antigen-specific antibody fragments.

As used herein "autoantigen / self-antigen", means a molecule produced and used by an individual self, which is recognized by an autoantibody, eliciting an immune response possibly leading to an autoimmune disease.

As used herein "A functional derivative of IAMT protein", means a derivative derivable from the respective natural form of IAMT by modification, e.g. by mutagenesis like amino acid substitution, deletion, insertion or addition, or by chemical modification, said derivative substantially showing biological activity by preventing or alleviating an autoimmune response either by decreasing or enhancing IAMT activity.

As used herein "desmethyl and/ or despropargyl derivative" means the compound of regard without the methyl and/ or propargyl group.

As used herein "molecule", means any chemical compound either synthetic or natural occurring, including DNA, RNA,

peptides, proteins or fragments thereof as well as small inorganic and organic compounds.

As used herein "patient" means an individual consulting a medical practitioner.

As used herein "regulator of IAMT activity", means a molecule affecting the basal activity of IAMT at any level. For example IAMT itself, IAMT agonists, catalyst, antagonists, gene expression enhancers or inhibitors, RNA stabilisers, inhibitors or activators of molecules interacting with IAMT.
As used herein, "sequence independent context", means that the sequence, surrounding the L-iso-aspartyl and/ or D-aspartyl residue(s), can be composed of virtually any of the 20 natural occurring amino acids or derivatives thereof, in a random order, producing a peptide or protein or a peptide like structure.

As used herein, "suitable promoter", means an inducible or constitutively active promoter operably linked to a coding region. The promoter is only transcribed under certain conditions, for example in certain tissues, cells or as a reaction to a certain disease possibly by induction through molecules generated as a result of the disease.

In one embodiment of the present invention the administration of a molecule with a regulatory effect on IAMT activity, within one or more cell types will enable prevention, treatment or alleviation of an autoimmune response or an autoimmune disease. Especially cell-types

associated with the immune system, such as B-cells, dendritic cells, macrophages, mast cells, monocytes, neutrophils, NK cells or T-cells are considered, most preferred are T-cells and antigen presenting cells, such as dendritic cells, macrophages and B-cells. Other cell-types of importance, are cells that become targets for an autoimmune attack by the immune system, such as, but not limited to, pancreatic β-cells, nerve cells, Schwann cells, mucus secretory cells such as goblet cells, salivary gland cells or other endocrine gland cells.

A humoral or cell mediated immune response directed toward a self-antigen/ autoantigen, is considered to be an autoimmune response. An autoimmune response often leads to an autoimmune disease. The present invention provide means for therapeutic interventions or disease prevention of autoimmune diseases such as, but not limited to, celiac disease, Crohns disease, insulin dependent diabetes mellitus, Grave's disease, multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus or ulcerative colitis.

We describe herein methods for identifying regulators of IAMT activity from candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs), which have a modulatory (*i.e.*, stimulatory or inhibitory) effect on, for example, expression or activity of IAMT.

Cell-based screening assays for IAMT have been described in WO 98/15647, either measuring the level of gene expression using a reporter protein, mRNA or protein levels with techniques generally know in the art. Furthermore a direct assessment of IAMT activity was described utilizing S-adenosyl-L-[methyl-^{3}H]-methonine, followed by measuring the incorporation of methyl-^{3}H into the substrate (L-iso-aspartyl) by fluorography. A similar technique measuring IAMT activity is described in the ISOQUANT kit from Promega utilizing a scintillation counter or HPLC.

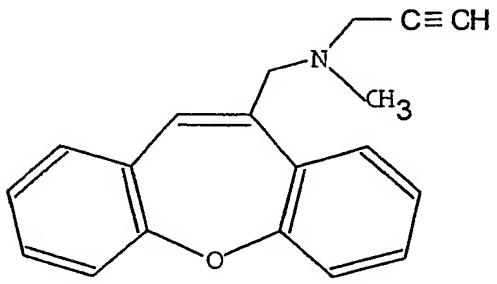
A test system for IAMT activity need not however utilise radioactivity or time-consuming HPLC techniques. The preferred test system is cell-based, containing L-iso-aspartyl and/ or D-aspartyl peptides and expressing IAMT. The cell, for example, can be a yeast cell, a cell of mammalian origin or a tissue section. A cell-free system can also be applied when testing compounds acting directly on IAMT, L-iso-aspartyl or D-aspartyl. The test system is contacted with the test compound and the ability of the test compound to regulate IAMT activity is determined by measuring substrate conversion utilizing an immunoassay. Antibodies, which recognize either L-iso-aspartyl or D-aspartyl in a sequence independent or sequence dependent context, constitute a part of the test system and will enable a fast determination of a compounds effect on IAMT activity. A reduced level of antibody binding, as compared to suitable controls, means a decrease in L-iso-aspartyl and/ or D-aspartyl containing peptides, which correlate with an increase in IAMT activity. Antibody binding can be assessed by techniques generally know in the art, for

example Western blot, ELISA, RIA, immuno-precipitation or histology.

The method for measuring IAMT activity as described above can be provided as a kit. This will include a suitable test system, for example a cell free system containing IAMT protein and L-iso-aspartyl and/ or D-aspartyl containing peptides or a cellular system (e.g. e-coli, yeast, mammalian cell-lines, primary cell cultures or tissue sections) containing and expressing endogenous (homologous) and/ or exogenous (heterologous) IAMT encoding nucleic acid. The expression can be coupled to an easy detectable reporter protein, such as, but not limited to, β-galactosidase, chloramphenicol acetyl-transferase (CAT), Green Fluorescent Protein, or luciferase. Furthermore the kit includes a context independent or dependent antibody recognizing a L-iso-aspartyl or D-aspartyl, and possibly a second antibody with specificity towards the first antibody. For competition measurements a synthetic or naturally occurring peptide containing one or more L-iso-aspartyl or D-aspartyl residues might be supplied either in a labelled or unlabelled form. The antibodies may be used with or without modifications. The antibodies may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like. Antibodies or synthetic peptides of the kit might be immobilised, preferably on a solid surface like a micro-tittre plate, possibly by conjugation to a suitable protein

carrier like BSA, thyroglobulin, ovalbumin or keyhole limpet hemocyanine.

A preferred embodiment of the present invention, is the use of N-(dibenz [b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine (Formula II) for the preparation of a composition for preventing, alleviating or treating an autoimmune response and/ or disease in a mammal.



Formula II
(CGP 3466B)

Metabolites of N-(dibenz [b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine are also covered in the present invention, specifically the N-desmethyl, N-despropargyl and N-desmethyl-despropargyl derivatives.

Moreover the invention is directed to the use of IAMT protein or a functional derivative thereof for the preparation of a composition for preventing, alleviating or treating an autoimmune response and/ or disease.

One way to gain control of an autoimmune disease could be through the use of compounds for the preparation of a pharmaceutical composition, which decrease T-cell proliferation and/ or autoantigen presentation on MHC II molecules, thereby preventing, alleviating or treating an autoimmune response. Preferably the compounds are chosen

among those identified by the the method for identifying regulators of IAMT activity, provided in the present invention, or among the oxepines described in the above or the IAMT protein or a functional derivative thereof.

Also contemplated are pharmaceutical compositions for prevention, alleviation or treatment of an autoimmune response and/ or disease, involving combination therapies comprising, administering an effective amount of IAMT protein, functional derivative thereof or a IAMT modulator in combination with other therapeutic agents. Other therapeutic agents can be, for example, anti-inflammatory drugs (e.g. NSAIDs, Phosphosugars or COX-2 inhibitors), anti-diabetes agents, immunotherapeutic agents, insulin-releasing agents (e.g. GLP-1, nateglinide, repaglinide, sulfonylurea, vasopressin), cytokines (e.g. interferons, interleukins, tumor necrosis factor, Fas ligand, cytokine antagonist (i.e. antibodies or receptors to TNF-α, IL-1, IL-6 or IL-12) or protease inhibitors (e.g. cysteine protease inhibitor, DPP IV antagonist, serine-protease inhibitor).

When administered to a patient, an IAMT protein, functional derivative thereof or a regulator of IAMT activity is preferably administered as a component of a composition that optionally comprises a pharmaceutically acceptable carrier, excipient or vehicle. In a preferred embodiment, these compositions are administered orally. Other administration routes may be, but are not limited to, depot injection, implantation, intracavitary, intramuscular, intravenous, nasal, subcutaneous, time-release mode or transdermal. The

pharmaceutical composition is formulated to be compatible with its intended route of administration.

Compositions for oral administration might require an enteric coating to protect the composition(s) from degradation within the gastrointestinal tract. In another example, the composition(s) can be administered in a liposomal formulation to shield the IAMT protein, functional derivative thereof or an IAMT modulator disclosed herein, from degradative enzymes, facilitate the molecule's transport in the circulatory system, and effect delivery of the molecule across cell membranes to intracellular sites.

Pharmaceutical compositions applicable in gene therapy approaches can also be used in accordance with the present invention to modulate the expression of an IAMT protein or an IAMT regulator (including IAMT antisense) and accordingly treat, alleviate or prevent an autoimmune response and/ or disease. Any of the methods for gene therapy available in the art can be used in accordance with the present invention. IAMT encoding nucleic acid sequences can be assessed through, but not limited to, Genbank accession nr. D13892, D25545, D25546, M60320, M26686, D11475, hereafter incorporated by reference.

A recipient's cells or heterologous cells can be engineered to express IAMT protein, IAMT regulator or a combination. The cells can be grown as an implant in an experimental animal or in tissue culture using techniques known in the art. Once altered genetically, the engineered cells can then be administered to a subject using procedures known in

the art. Alternatively, one can use gene therapy to transfect the recipient's cells *in vivo*.

The present invention encompasses expression vectors comprising a nucleic acid sequence encoding an IAMT protein or an IAMT regulator of the invention. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant construct. Alternatively, vectors can be used, which selectively target a tissue or cell type, *e.g.* viruses that infect antigen presenting cells or T-cells. Further specificity can be realized by using a tissue-specific or cell-specific promoter in the expression vector.

In one embodiment, an expression vector containing a nucleic acid sequence encoding an IAMT protein or an IAMT regulator to be introduced for purposes of gene therapy, comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid sequence can be controlled using an appropriate inducer or inhibitor of transcription.

In another embodiment, the vector contains a promoter, which expresses the cloned construct constitutively. The promoter can be down-regulated using a suppressor molecule. Alternatively, the vector contains a promoter, such that an inducing molecule initiates or increases expression of the cloned nucleic acid sequence.

In a preferred embodiment, the vector contains a specific promoter. Such a promoter can for example restrict expression to occur in a specific tissue or organ, such as,

but not limited to, skin, muscle, intestine, lung, cartilage, bone, brain or certain areas of the brain, pancreas, liver, kidney or thymus. Specific cell types can also be a target for such a promoter, for example cells associated with the immune system, such as B-cells, dendritic cells, macrophages, mast cells, monocytes, neutrophils, NK cells or T-cells, antigen presenting cells, such as dendritic cells, macrophages and B-cells. Other cell-types, such as, but not limited to, pancreatic β-cells, Schwann cells, epithelia cells, mucus secretory cells such as goblet cells, salivary gland cells or other endocrine gland cells. A vector containing a disease-specific promoter, such that expression is largely limited to diseased tissues or tissues surrounding diseased tissues is also a possibility. A disease specific promoter could be controlled through certain cytokines, antibodies or other molecules released as reaction to a certain disease. Formulations of nucleic acid sequences for gene therapeutic methods can be, but are not limited to, naked DNA, nucleic acid sequence encapsulated into liposomes or liposomes combined with viral envelope receptor proteins, DNA coupled to a polylysine-glycoprotein carrier complex, and nucleic acid precipitants.

The present invention additionally encompasses methods of diagnosing or assessing an individuals risk developing an autoimmune disease, associated with irregularities connected to IAMT.

The gene encoding IAMT protein is known to contain polymorphisms, where at least one has been shown to result in different enzyme activities (David, Szumlanski, DeVry,

Park-Hah, Clarke, Weinshilboum, and Aswad 1997; Tsai and Clarke 1994).

A study connecting such genetic polymorphisms to autoimmunity is conducted. The known exon polymorphisms of the human IAMT1 (PCMT1) gene, are amino acid 22 Ile/Leu, amino acid 119 Val/Ile and amino acid 205 Lys/Arg, their connection to autoimmunity is determined, as well as new exon or intron polymorphisms, which emerge through the study. A new polymorphism is reported below.

In one embodiment the results from such a study are the basis for a method for diagnosing or assessing an individual risk developing an autoimmune disease. Methods for determination of genetic polymorphism in genomic DNA include, but are not limited to, direct comparison of sequences of different genomes, pulsed field gel electrophoresis, alterations in restriction enzyme cleavage patterns or polymerase chain reaction with designed primers. The screening of genetic polymorphism in the IAMT gene can be performed on any biological material containing genomic DNA, for example blood, erythrocytes, hair, saliva or tissue samples.

For example, the invention includes a method for diagnosing an autoimmune disease or assessing an individual's risk of developing diabetes (type I and type II diabetes), comprising detecting the IAMT polymorphy

22132

AGATCCGCCGCTCGAAACAG***C***TGACCCAGCGACGACTGCGG

AGATCCGCCGCTCGAAACAG***G***TGACCCAGCGACGACTGCGG

at position 22132 of the PCMT1 (IAMT) gene in a biological sample from a patient versus a control.

In another embodiment of the present invention irregularities connected with IAMT gene transcription level, protein level or activity are utilized for diagnosing or assessing an individuals risk of developing an autoimmune disease.

For quantitative determination of IAMT gene transcription level in an individual, the amount of IAMT mRNA in a sample can be measured utilizing techniques generally know in the art which include for example rtPCR, micro arrays or Northern blot techniques. A decreased IAMT gene transcription level compared to a control, for example a group of healthy individuals, indicates a risk of autoimmunity or possible diagnosis of autoimmunity.

The IAMT protein level indirectly reflects gene transcription level as well as mRNA stability. Techniques for measuring proteins levels are generally known in the art and include for example Western blot analysis, ELISA, RIA, immuno-precipitation, histology, micro arrays and the like.

A method for quantification of IAMT activity, utilizing antibodies, which recognize L-iso-aspartyl or D-aspartyl in a sequence independent or dependent context, has already been disclosed above, and can also be applied for diagnostic means. The level of L-iso-aspartyl or D-aspartyl containing peptides present in a sample, provided from an individual to be diagnosed, can be used to assess the

activity of endogenous IAMT in the individual. Other methods to assess IAMT activity can also be utilized in relation to diagnosis of an autoimmune disease, for example the method described in the ISOQUANT kit from Promega. A decreased IAMT level compared to a control, for example a group of healthy individuals, indicate a risk of autoimmunity or possible diagnosis of autoimmunity.

Preferably any of theabove described measurements performed for diagnosis are determined against suitable controls, e.g. healthy individuals or cell lines where IAMT baseline expressions are known.

The diagnostic measurements can be performed on biological samples such as, but not limited to, human body fluids (e.g. blood, serum or urine samples) or extracts from cells or tissue samples. Another possibility is to isolate specific cell types from blood or tissue samples, where IAMT play a role in connection with autoimmunity, such as T-cells or antigen presenting cells. Cells circulating in the blood can be isolated using FACS. Cells can also be cultured from an area affected by an autoimmune response, followed by selection for one or more specific cell types, e.g. macrophages, dendritic cells or the like.

The invention will be further illustrated by the following examples and the accompanying drawings in which:-

Figure 1 A-C shows reverse-phase HPLC traces of MBP_{87-99} upon AEP incubation. The MBP_{87-99} peptide VHFFK**N**IVTPRTP (Asn form) is incubated in the presence or absence of 1 U/ml pig kidney AEP at 30°C before analysis by reverse-phase HPLC.

Un-modified substrate (VHFFKNIVTPRTP) elutes at approximately 17.3 min. Generated cleavage products eluting at 13.6 and 15.0 minutes are identified as IVTPRTP and VHFFKN respectively. (A) MBP_{87-99} peptide in the absence of AEP. (B) MBP_{87-99} peptide incubated 4h with AEP. (C) MBP_{87-99} peptide incubated 20h with AEP.

Figure 2 A-B shows reverse-phase HPLC traces of isomerised MBP_{87-99} upon AEP incubation. The MBP 87-99 peptide VHFFK**Diso**IVTPRTP (iso-Asp form) is incubated in the presence (B) or absence (A) of 1 U/ml pig kidney AEP at 30°C for 20h before analysis by reverse-phase HPLC.

Figure 3 is a table showing predicted and observed masses obtained by mass-spectroscopy of peptides incubated in the absence or presence of 1 U/ml pig kidney AEP at 30°C.

Figure 4 shows results obtained in Example 2 in graph form.

Examples

Example 1: Iso-Asp and D-Asp formation abrogates cleavage by asparginyl endopeptidase potentially triggering an autoimmune response.

Asparginyl endopeptidase (AEP) or legumain (EC 3.4.22.34) is a cysteine endopeptidase of the peptidase family C13. AEP has recently been shown to be implicated in protein processing for the MHC class II system, and specifically to play a key role in the processing of self antigens (Antoniou et al 2000; Hewitt et al 1997; Manoury et al 1998; Manoury 2001).

Peptides

Peptides used in the experiment are MBP_{87-99} peptide VHFFK**N**IVTPRTP, VHFFK**Diso**IVTPRTP, VHFFKD_DIVTPRTP and Insulin B-chain FV**N**QHLCGSHLVEALYLVCGERGFFYTPKT, FV**Diso**QHLCGSHLVEALYLVCGERGFFYTPKT and FVD_DQHLCGSHLVEALYLVCGERGFFYTPKT.

The peptides are synthesised using Fmoc chemistry, the purity of the peptides are >95% as judged by RP HPLC analysis.

Peptide hydrolysis by AEP

Peptides are incubated at 10 µM with 1 mU/ml AEP (from pig kidney) in 200 µl 39.5 mM citric acid, 121 mM $Na_2H_2PO_4$, pH 5.8 containing 1 mM EDTA, 1mM DTT and 0.01% CHAPS. Incubation is performed for up to 20 h at 30°C. Samples are removed at intervals and the reaction stopped by adding an equal volume of 200 mM Tris-HCl, pH 8.5 and immediately frozen at -80°C. Ten µM peptide solution is incubated without AEP addition as control.

The products of the hydrolysis are separated by reversed phase HPLC on Vydac C_{18} 5 µm column (250 mm x 4.6 mm, Cat. No. 218TP54).

Two gradients are applied to separate intact and hydrolysed products of MBP_{87-99} peptide and insulin B chain. For MBP_{87-99} a linear gradient from 0-50 % (v/v) acetonitrile containing 0.1 % trifluoroacetic acid is applied over 20 min at 1.4 ml/min (Figure 1 and 2).

For insulin B-chain a linear gradient from 0-50 % (v/v) acetonitrile containing 0.1 % trifluoroacetic acid is applied over 20 min at 1.4 ml/min.

The column effluent is monitored at 214 nm and peak areas are integrated using the Waters Millenium software. For each time point, the areas under the peaks representing products and unchanged substrate are recorded.

Analysis of cleavage products

Cleavage products generated through AEP cleavage are identified by mass spectrometric analysis. Mass spectrometry is done using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) in a Reflex III spectrometer (Bruker Analytical Systems Inc., Billerica, MA, USA). Material is lyophilised and re-dissolved in 20 µl of 30% acetonitrile (v/v) containing 0.15 % TFA (v/v). A 2 µl aliquot is mixed with 2 µl of saturated α-cyano-4-hydroxycinnaic acid as matrix in the same solvent, 0.7 µl of this mixture is spotted onto the target plate. Samples are evaporated and analysed in reflector and linear mode at 22.5 kV. The spectra are averaged from 50-100 laser-beam shots and calibrated externally with proteins of known masses. MALDI-TOF is performed both on crude cleavage mixtures and purified cleavage products (collected by pooling relevant HPLC-fractions).

Mass spectrometric analysis of the products confirm that AEP cleaved MBP and insulin B-chain at asparginyl residues. Furthermore the results show that isomerisation and

racemisation blocks attack by AEP at otherwise susceptible sites (Figure 3). Blocking AEP cleavage has recently been shown to ensure presentation of MBP epitopes (on MHCII), which would otherwise escape presentation (Manoury 2001). This means that introduction of a D-Asp, iso-Asp or D-iso-Asp residue within a self protein can lead to presentation on MHCII, potentially leading to T-cell proliferation and autoimmunity.

Example 2

In Vivo Validation of CGP3466B for Treatment of Autoimmune Diseases and Diabetes

Aim

The aim of the present study is to validate the compound CGP3466B for prevention or treatment of autoimmune disease (IDDM)and to correlate the expression of IAMT to the effect of the drug on disease progression and severity.

Study of the Effect of CGP3466B in the BB/OK Rat Model

Animal Model

The animal model used for the validation of compounds modulating IAMT activity for the treatment of autoimmune diseases should preferentially be spontaneous and affect the IAMT gene.

To identify a suitable animal model the location of the IAMT gene was compared to the location of autoimmune susceptibility locuses within known spontaneous autoimmune models. One suitable model was identified; the spontaneous diabetic BB/OK rat model. The BB/OK rat is a spontaneous diabetic animal developing diabetes at the age of 3-6 months. The genetic locus responsible for the diabetic

phenotype of this rat strain has been mapped to a site on chromosome 6 spanning 3 cM and encompassing the IAMT gene.

The diabetes syndrome of the BB/OK rat resembles human type 1 (insulin dependent) diabetes, both in terms of clinical and histological features and the prevalence of autoantibodies to GAD. Moreover, the BB/OK rat has the phenotypic characteristics expected for a IAMT deficient animal; increased cellular apoptosis, hyperproliferation of T-lymphocytes and autoimmunity.

Study Design

The prophylactic effect of 10-(N-methyl-N-propargyl-amino) methyldibenz [b,f] oxepine (CGP3466B - Formula II) on preventing or ameliorating diabetes in the BB/OK rat was studied. CGP3466B was tested in 3 different doses.

Seventy rats were randomly assigned to one of the following 5 treatment groups: A) SHR rats (non-diabetic), saline only; B) BB/OK rats, saline only; C) BB/OK rats saline + 14µg/kg CGP3466B; D) BB/OK rats saline + 140 µg/kg CGP3466B; and E) BB/OK rats saline + 500 µg/kg CGP3466B. CGP3466B was administered subcutaneously (injections, 200 µl per rat are given three times per week (Monday, Wednesday and Friday)). Treatment starts when animals are 5 weeks old and is continued until animals are 20 weeks old. All rats will be sacrificed at week 20.

Throughout the study rats are bled each second week (to monitor blood glucose, evaluate autoantibody levels and target gene expression levels). Serum and blood cells are prepared from the blood and stored at -80°C. Disease severity is assessed by histological assessment of insulitis (infiltration and destruction of pancreatic islets).

The effect of CGP3466B on the expression of the target protein is monitored by measuring gene transcription levels in pancreatic cells and lymphocytes by quantitative RT PCR using SYBR-Green. In addition target protein expression and activity levels in pancreatic cells or lymphocytes) is also analyzed by an immunoassay specific for the gene product as well as using an enzyme activity assay. Moreover the presence of antibodies against GAD is assessed.

Care of Animals

Animals are maintained under standard conditions (non-specific pathogen free) with free access to food and water, and they are cared for according to the guidelines of the local animal care committee.

Appearance and activity is monitored daily. Animals are weighed each second week.

Throughout the study rats are bled each second week (to monitor blood glucose, evaluate autoantibody levels and

target protein expression levels). Serum aliquots are stored at -20°C until use.

Determination of Blood Glucose, Diagnosis of Diabetes

The presence of diabetes is determined by visual inspection and is confirmed by measurements of blood glucose. Animals having glucose levels higher than 300 mg/dl on two consecutive dates will be considered diabetics.

Animals are terminated at the onset of clinical diabetes (blood glucose >300 mg/dl). The rest of the animals are killed at week 20.

Histology and Immunohistochemistry of Pancreatic Islets

Animals are killed using CO_2 or ether anesthesia for islet histology. Half of each pancreas is frozen for target gene expression studies. The other half is divided in two. One quarter of the pancreas is used for histology; it is fixed in Bouin's solution for 72 h and placed in 70% alcohol. After treatment with 100% alcohol the sections are embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin.

Histological examination of pancreatic islets is performed in a blinded fashion by a pathologist unaware of the status and/or treatment of the animals. The degree of mononuclear cell infiltration is graded as follows: 0, no infiltrate; 1 periductular infiltrate; 2, periislet infiltrate; 3, intraislet infiltrate; and 4, intraislet infiltrate associated with β-cell destruction.

At least 10 islets are counted for each rat. The mean score for each rat is calculated by dividing the total score by the number of islets examined.

The other quarter is used for immunohistochemical analysis; pancreata are quick-frozen in TissueTek (Sakura, Torrance, California, USA). Tissue sections (8µm) are stained with antibodies to rat Insulin (RDI-TRK2IP10-D6C4, Research Diagnostics Inc.), CD4 (W3/25, Cedarlane Labs), CD8 (OX-8, Cedarlane Labs), MHC Class I and MHC Class II (Cedarlane Labs). Sections are stained with an appropriate species-specific, biotinylated secondary antibody, a streptavidin-horseradish peroxidase complex and diaminobenzidine. Slides are counterstained with hematoxylin and embedded in AquaMount (Fischer Scientific Co., Pittsburgh, Pennsylvania, USA).

Analysis of Target Protein Expression in Pancreatic Cells using Real-time RT-PCR (Performed at NORDIC)

Total RNA is isolated from the pancreata of non-diabetic SHR rats and from BB/OK rats treated with the compound or placebo and reverse-transcribed. cDNA is synthesized from 0.5-1.0 µg RNA.

RT-PCR is carried out using a Light-Cycler™ System (Roche), which allows amplification and detection (by fluorescence) in the same tube, using a kinetic approach. Light-Cycler PCR reactions are set up in microcapillary tubes using 5 µl cDNA with 5 µl of a 2× SYBR Green I (Roche Molecular Biochemical's, Wittwer et al., 1997) master mix containing upstream and downstream PCR primers, $MgCl_2$ and SYBR Green.

After each elongation phase, the fluorescence of SYBR Green (a dye that binds double-stranded DNA giving a fluorescent signal proportional to the DNA concentration) is measured at a temperature 1°C below the determined melting point for the PCR product being analyzed. This excludes primer-dimers, which melt at lower temperature, from the measurement. The fluorescence level is thus quantitated in real-time, allowing the detection and display of the log-linear phase of amplification as it happens. Light-cycler quantification software v 1.2 is used to compare amplification in experimental samples during the log-linear phase to the standard curve from the dilution series of control cDNA. For each primer pair used, conditions are optimized so that melting curve analysis shows a single melting peak after amplification indicating a specific product. Transcription levels are normalized to the RNA of ubiquitous housekeeping proteins.

Analysis of Target protein Expression in Pancreatic Cells using ELISA

The content of target protein within the cytosol of pancreatic β-cells (or lymphocytes) is assessed by an ELISA.

Analysis of Target Protein Activity in Pancreatic Cells using enzyme assay

The activity of the target protein is determined in the cytosol of pancreatic cells by applying an enzyme assay.

FACS Analysis of Leukocytes

Blood from rats is analyzed at expected day of onset (around day 90). 5-10 rats from each group.
Blood is drawn from each rat. Leukocytes are isolated from blood using centrifugation. Cells are stained and fixed. FACS analysis is then performed over one or two days using:
a) T cell activation markers: Double-stain with R73 + OX 39. (TCR and CD25, phenotype changed in BB.6S) or
b) T cell G1 cycle analysis (T cell -proliferation): Separate the T lymphocytes using MACS-column and anti-CD4ab, stain nucleus using propidium iodine and perform FACS analysis.

Assessment of Autoantibodies against Glutamic Acid Decarboxylase

Autoantibodies against glutamic acid decarboxylase (GAD) are detectable in most but not all newly diagnosed type I diabetic patients (Bækkeskov et al 1990). GAD autoantibodies have proved to be an early indicator of IDDM

and appear to be more predictive than insulin autoantibodies (Aanstoot et al 1994).

Prevalence and level of autoantibodies against GAD are tested for in BB/OK rats using a direct ELISA and compared between treatment groups. The measurement of GAD autoantibodies is performed essentially as described by Ziegler et al 1994.

Results

The results as at week 13 of the study are compiled in table 1 below.

Table 1: Analysis of the Animal study as of day 98

	Dose of CGP3466B				
Age at Onset	0µg/kg	14µg/kg	140µg/kg	500µg/kg	Total
72			1		1
73	1				1
74					
75					
76		2			2
77					
78	1				1
79					
80	1				1
81					
82					
83			1		1
84		2			2
85					
86	1	1	1		3
87			1	1	2
88					
89		1		1	2
90					
91	1		1		2
92					
93	1		2		3
94					
95		1			1
96					
97					
98	1		1		2
Total Diabetics	7	7	8	2	24
Total Animals	15	15	15	15	60
% Diabetics	46.67%	46.67%	53.33%	13.33%	40.00%
Mean age at diabetes onset	85.57	84.29	87.88	88.00	86.17
SD age at diabetes onset	9.00	6.80	7.97	1.41	7.42

In the groups treated with placebo (saline only) or the two lowest doses of compound the incidence of diabetes is 40-50 % as expected for untreated BB/OK rats, however the incidence is significantly lower in the 500 µg/kg group (13%).

In addition the age of onset of diabetes appears to be postponed in a dose-dependent manner (the higher the dose the later the onset of diabetes).

Figure 4 shows "survival curves" for the study comparing the high treatment group (500 µg/kg) with the vehicle group (0 µg/kg, figure4 A) and the vehicle group pooled with the two low-treatment groups (0 µg/kg + 14 µg/kg +140 µg/kg), figure 4B. The rate of diabetes development is significantly higher in the vehicle group and the 14 µg/kg and 140µg/kg treatment groups as compared to the high dose group.

The Hazard ratio is 4.3 and 4.6 respectively, figure 4 A and B (as compared to the vehicle group alone or the vehicle group and the low-dose treatment groups respectively), meaning that the rate of diabetes development is more than four-fold higher in the animals which have not been treated with the high dose of CGP3466B. Thus these data show that compounds like CGP3466B may be used to treat or prevent autoimmune diseases.

Example 3: SNP analysis of the PCMT1 gene in human genomic DNA samples from families affected with IDDM.

Methods

A 100 kb fragment of contig NT_023451 was used for the design of primers producing PCR fragments spanning all protein-encoding exons and a promotor fragment.

PCR primers were designed for the amplification of genomic PCR fragments spanning the promotor and exon regions of PCMT1. Primers and fragment characteristics are described in figure X.

PCR fragments were produced on genomic DNA from persons from families affected by diabetes type I, using Platinum Pfx polymerase with various concentration of PCR enhancer (Invitrogen). Strong, single PCR fragments were obtained with primers for exon0, exon1, exon2, exon3 and exon 5, exon6. The promotor fragment was weaker, but showed an acceptable band. Exon4 was most difficult to amplify but after several trial with different primers we succeeded in amplifying a small band spanning the complete exon sequence.

PCR fragments for the promotor, exon0, exon3 and exon5 were precipitated and sent to MWG-Biotech, Germany, for sequencing. Only 30-50% of the samples resulted in high quality sequences. These sequences were of variable quality and length and were usually drastically shorter than the theoretically possible 700 bases per read.

Best sequences were obtained for exon3 and exon5. Exon0 and promotor were intermediate, whereas exon2 and exon6 had a lot of sequence ambiguities (read as "N").

The identity of the fragments as PCMT1 genomic fragments was confirmed by using the BLAST 2 software, which compares two single sequences.

DNA from ten control and ten patient samples was then used to amplify and sequence fragments of the promotor, exon0, exon3 and exon5. No single nucleotide changes were observed in the coding region of the three exons, whereas intron sequences varied a lot or were ambiguous.

Intriguingly, the promotor fragment revealed a novel SNP at position 22132 of the 100000 bp reference contig.
The sequence environment is presented below:
AGATCCGCCGCTCGAAACAGCTGACCCAGCGACGACTGCGG
AGATCCGCCGCTCGAAACAGGTGACCCAGCGACGACTGCGG

Underline: PvuII recognition site.

The SNP is a C->G transversion and is detectable with the restriction enzyme PvuII.
The PvuII recognition site is destroyed by the C->G transversion.

Homozygous "normal" individuals yield two promotor fragments of 565 and 392 base pairs.
Homozygous "normal" individuals yield the original promotor fragment of 957 base pairs.
Heterozygous individuals yield all three promotor fragments.

Samples were re-evaluated by PvuII restriction.

In total 21 samples from persons affected by diabetes type I and eight healthy members from the same group of families were evaluated (Figure 6).
This SNP might be "silent", because it occurred in healthy and sick individuals. However, no control for association of this SNP with these families has been made, opening the theoretical possibility that this SNP increases the risk for diabetes type I. This would have to be confirmed by analysis of DNA from independent families. There are

several transcription factors that have potential binding sites at this SNP site including AP1, AP4 and Lmo2. At present it is unknown whether the point-mutation abolishes the biding of these. Alterations in their binding pattern might affect PCMT1 expression and thereby potentially the capacity of cells to repair isomerised proteins.

References

Antoniou,A.N., Blackwood,S.L., Mazzeo,D., Watts,C., 2000. Control of antigen presentation by a single protease cleavage site. Immunity. 12. (4), 391-398.

David,C.L., Szumlanski,C.L., DeVry,C.G., Park-Hah,J.O., Clarke,S., Weinshilboum,R.M., Aswad,D.W., 1997. Human erythrocyte protein L-isoaspartyl methyltransferase: heritability of basal activity and genetic polymorphism for thermal stability. Arch Biochem Biophys 346, 277-286.

Doyle,H., Gee R., Mamula M. 2001. Post-translational protein modification of self-proteins leads to loss of tolerance. Abstract C8 Wed.3.30/627 at the 11th International Congress of Immunology in Stockholm, Sweden 2001.

Doyle,H.A., Mamula,M.J., 2001. Post-translational protein modifications in antigen recognition and autoimmunity. Trends Immunol 22, 443-449.

Hewitt,E.W., Treumann,A., Morrice,N., Tatnell,P.J., Kay,J., Watts,C., 1997. Natural processing sites for human cathepsin E and cathepsin D in tetanus toxin: implications for T cell epitope generation. J Immunol 159, 4693-4699.

Huebscher,K.J., Lee,J., Rovelli,G., Ludin,B., Matus,A., Stauffer,D., Furst,P., 1999. Protein isoaspartyl methyltransferase protects from Bax-induced apoptosis. Gene 240, 333-341.

EP patent 0726265, Zimmermann K. Betschart C.,10-Aminoaliphatyl-dibenz(b,f)oxepins with antineuro-degenarative activity, CIBA GEIGY AG (CH), 1996.

Kim,E., Lowenson,J.D., MacLaren,D.C., Clarke,S., Young,S.G., 1997. Deficiency of a protein-repair enzyme results in the accumulation of altered proteins, retardation of growth, and fatal seizures in mice. Proc. Natl. Acad. Sci. U.S.A 94, 6132-6137.

Macchi,B., Matteucci,C., Nocentini,U., Caltagirone,C., Mastino,A., 1999. Impaired apoptosis in mitogen-stimulated lymphocytes of patients with multiple sclerosis. Neuroreport 10, 399-402.

Mamula,M.J., Gee,R.J., Elliott,J.I., Sette,A., Southwood,S., Jones,P.J., Blier,P.R., 1999. Isoaspartyl post-translational modification triggers autoimmune responses to self-proteins. J Biol. Chem. 274, 22321-22327.

Manoury,B., Hewitt,E.W., Morrice,N., Dando,P.M., Barrett,A.J., Watts,C., 1998. An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. Nature 396, 695-699.

Manoury, B. 2001. Antigen processing and presentation in the MHC class II pathway; Peptide generation. Oral presentation S 2.3.1 at the 11th International Congress of Immunology in Stockholm, Sweden 2001.

PCT application WO 98/15647 Fuerst P., Modulation of apoptosis, CIBA GEIGY AG (CH), 1998.

PCT application WO 01/13110 Christgau S., Cloos P, Specific autoimmune reactions against isomerised/optically inverted epitopes: application for diagnosis of autoimmune diseases, Osteometer Biotech As (DK), 2001.

Rodenburg,R.J., Raats,J.M., Pruijn,G.J., van Venrooij,W.J., 2000. Cell death: a trigger of autoimmunity? Bioessays 22, 627-636.

Stadtman,E.R., 1988. Protein modification in aging. J Gerontol. 43, B112-B120.

Stekman,I.L., Blasini,A.M., Leon-Ponte,M., Baroja,M.L., Abadi,I., Rodriguez,M.A., 1991. Enhanced CD3-mediated T lymphocyte proliferation in patients with systemic lupus erythematosus. Arthritis Rheum 34, 459-467.

Tsai,W., Clarke,S., 1994. Amino acid polymorphisms of the human L-isoaspartyl/D-aspartyl methyltransferase involved in protein repair. Biochem Biophys. Res Commun. 203, 491-497.

Vratsanos,G.S., Jung,S., Park,Y.M., Craft,J., 2001. CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: a model to explain spontaneous T cell activation in lupus. J Exp. Med 193, 329-337.

Young,A.L., Carter,W.G., Doyle,H.A., Mamula,M.J., Aswad,D.W., 2001. Structural integrity of histone h2b in vivo requires the activity of protein l-isoaspartate o-methyltransferase, a putative protein repair enzyme. J Biol Chem 276, 37161-37165.

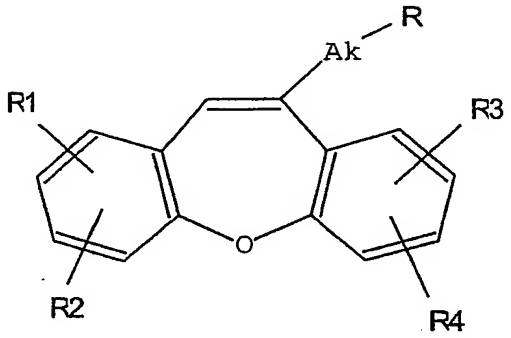
Zang,Y.C., Kozovska,M.M., Hong,J., Li,S., Mann,S., Killian,J.M., Rivera,V.M., Zhang,J.Z., 1999. Impaired apoptotic deletion of myelin basic protein-reactive T cells in patients with multiple sclerosis. Eur. J Immunol 29, 1692-1700.

Claims

1. The use of a regulator of L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT) activity for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

2. The use of a 10-aminoaliphatyl-dibenz[b,f]oxepine for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

3. A use according to claim 2, wherein the 10-aminoaliphatyl-dibenz[b,f]oxepine is of the general formula I:



Formula I

wherein Ak is a divalent aliphatic radical,
R is an amino group that is unsubstituted or mono- or di-substituted by monovalent aliphatic and/or araliphatic radicals or disubstituted by divalent aliphatic radicals, and

R1, R2, R3 and R4 are each, independently of the others, hydrogen, lower alkyl, lower alkoxy, halogen or trifluoromethyl.

4. A use as claimed in claim 3, wherein ak is methylene, R is amino; phenyl-C_1-C_4 alkylamino unsubstituted or substituted by C_1-C_4 alkyl, C_1-C_4 alkoxy halogen having an atomic number of up to and including 35 and/or by trifluoromethyl; N-phenyl- C_1-C_4 alkyl-N- C_1-C_4 alkyl-mino unsubstituted or substituted by C_1-C_4 alkyl C_1-C_4 alkoxy, halogen having an atomic number of up to and including 35 and/or by trifluoromethyl; or C_2-C_7 alkenylamino, C_2-C_7 alkynylamino, N- C_2-C_7 alkenyl-N- C_1-C_4-alkylamino or N- C_1-C_4 alkynyl-N- C_1-C_4 alkylamino, and R_1, R_2, R_3 and R_4 are each, independently of the others, hydrogen, C_1-C_4 alkyl C_1-C_4 alkoxy, halogen having an atomic number of up to and including 35 or trifluoromethyl, or a salt thereof.

5. A use according to claim 2, wherein the compound is
N-(dibenz[b,f]oxepin-10-ylmethyl)-N-prop-2-ynylamine,
N-(1-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,
N-(7-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,
N-(8-methoxy-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,
N-(8-tert-butyl-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,
N-(6-chloro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,

N-(1-fluoro-dibenz[b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine,
N-(7-chloro-dibenz[b,f]oxepin-10-methyl)-N-methyl-N-prop-2-ynylamine or a salt of any one thereof.

6. A use according to claim 2, wherein the compound is N-(dibenz [b,f]oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine.

7. A use as claimed in any preceding claim, wherein the effect of the compound to be administered is an up-regulation of IAMT activity.

8. A use according to claim 1 or claim 2, wherein said composition comprises a compound as defined in any of the claims 1 to 7, together with at least one pharmaceutically acceptable carrier and/ or excipient.

9. The use of IAMT or a functional derivative thereof for the preparation of a composition for the prevention, treatment or alleviation of an autoimmune response and/ or disease in a mammal.

10. The use of a IAMT encoding nucleic acid sequence or a functional derivative thereof for the use in preventing, alleviating or treating an autoimmune disease in a mammal.

11. A use according to claim 10, wherein the IAMT encoding nucleic acid sequence is provided in an expression vector comprising a suitable promoter for expression of IAMT.

12. A method for preventing, treating or alleviating an autoimmune response and/ or disease in a mammal comprising regulating L-Isoaspartyl (D-Aspartyl) O-Methyltransferase (IAMT) activity by administering a molecule with such a regulatory effect.

13. A method according to claim 12, wherein the regulation occurs within one or more cell types included in antigen presenting cells, T-cells or cells that become targets for an autoimmune attack by the immune system.

14. A method according to claim 13, wherein the regulation occurs within pancreatic ß cells, or nerve cells.

15. A method according to any one of claims 12 to 14, wherein the regulation of IAMT activity is an up-regulation.

16. A method of diagnosing an autoimmune disease or assessing an individuals risk of developing an autoimmune disease, comprising screening for genetic polymorphisms in the IAMT gene.

17. A method as claimed in claim 16, wherein said genetic polymorphism comprises the sequence:
AGATCCGCCGCTCGAAACAGGTGACCCAGCGACGACTGCGG

18. A method of diagnosing an autoimmune disease or assessing an individuals risk of developing an autoimmune disease, comprising quantification of IAMT on gene transcription level, protein level or activity, in a biological sample from a patient versus a control.

Figure 1

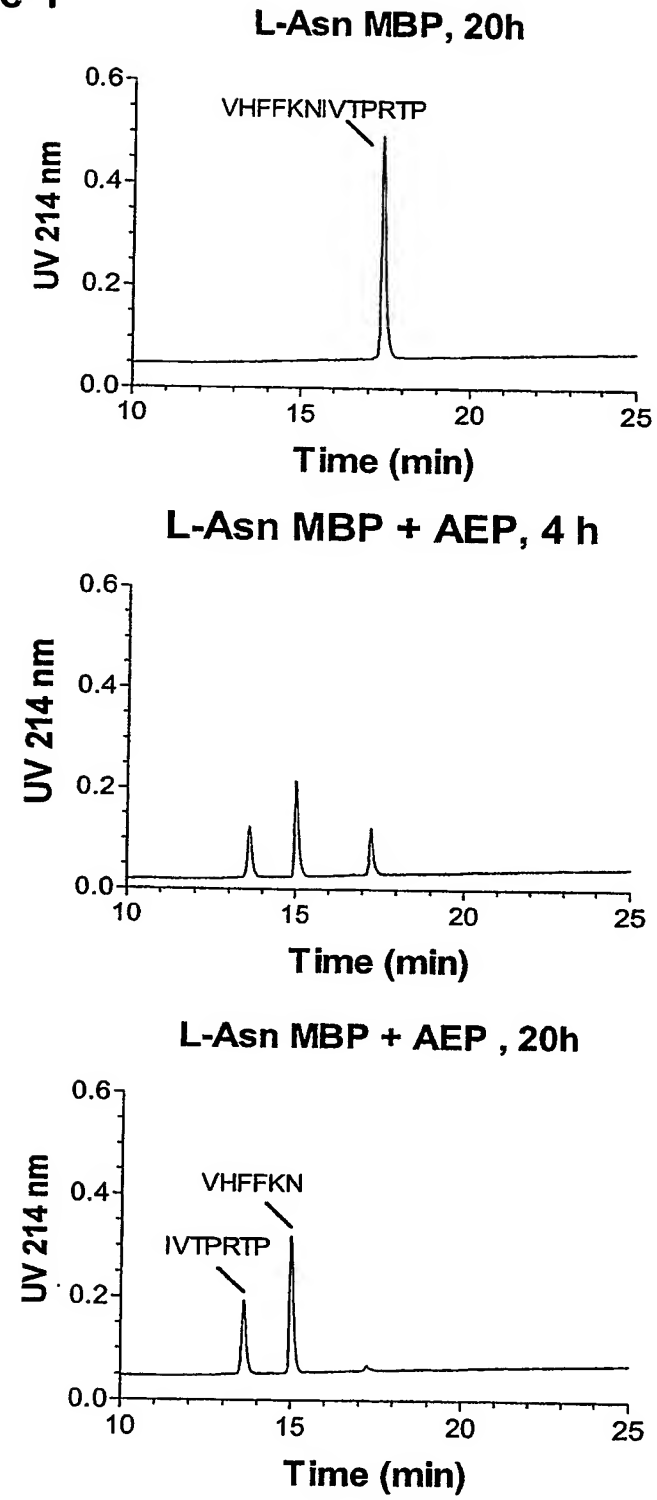
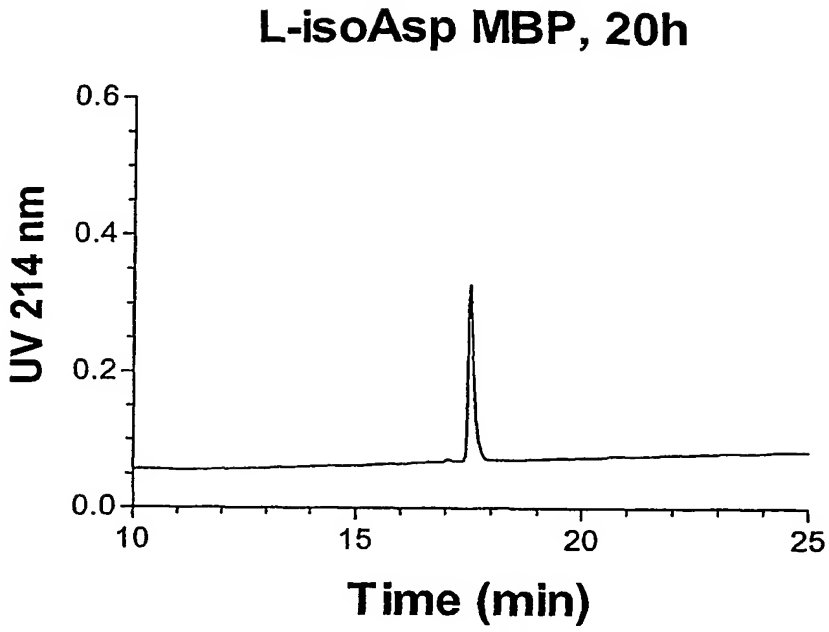

L-Asn MBP, 20h
A.
VHFFKNIVTPRTP
UV 214 nm
Time (min)
L-Asn MBP + AEP, 4 h
B.
UV 214 nm
Time (min)
L-Asn MBP + AEP , 20h
C.
VHFFKN
IVTPRTP
UV 214 nm
Time (min)

Figure 2


L-isoAsp MBP, 20h
UV 214 nm
0.6
0.4
0.2
0.0
10
15
20
25
Time (min)

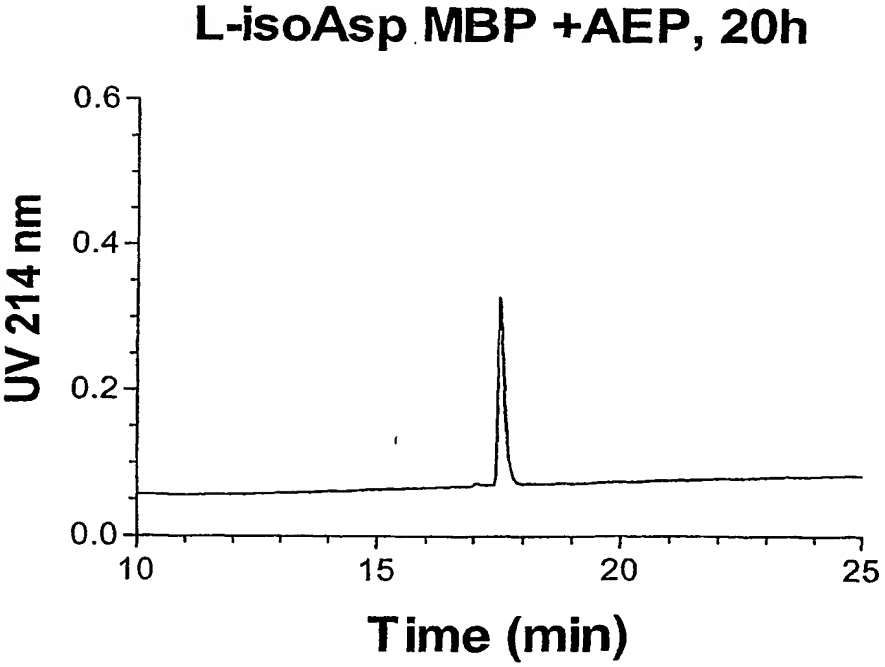
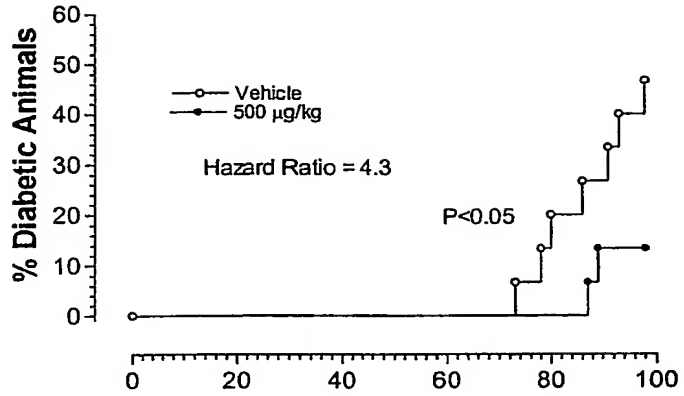

L-isoAsp MBP +AEP, 20h
UV 214 nm
0.6
0.4
0.2
0.0
10
15
20
25
Time (min)

Figure 3

Table: Isomerisation and racemisation blocks AEP action on autoantigens

Asn form of MBP_{87-99} peptide		**Predicted mass**	**Observed mass**
No enzyme			
	VHFFK**N**IVTPRTP	1555.87	1555.57
+ AEP			
	VHFFK**N**IVTPRTP	1555.87	1555.57
	IVTPRTP	783.47	783.39
	VHFFK**N**	791.42	791.28
Iso-Asp form of MBP_{87-99} peptide		**Predicted mass**	**Observed mass**
No enzyme			
	VHFFK**Diso**IVTPRTP	1556.86	1556.40
+ AEP			
	VHFFK**Diso**IVTPRTP	1556.86	1556.36
D-Asp form of MBP_{87-99} peptide			
No enzyme			
	VHFFKD_DIVTPRTP	1556.86	1556.80
+ AEP			
	VHFFKD_DIVTPRTP	1556.86	1556.85
Asn form of Insulin B chain			
No enzyme		3427.68	3428.20
+ AEP		3427.68	3428.27
	EHLCG...	3068.51	3068.50
	FVN	379.20	378.19
Iso-Asp form of Insulin B chain			
No enzyme		3428.68	3429.42
+ AEP		3428.68	3428.96
D-Asp form of Insulin B chain			
No enzyme		3428.68	3429.24
+ AEP		3428.68	3429.16

A.



B.

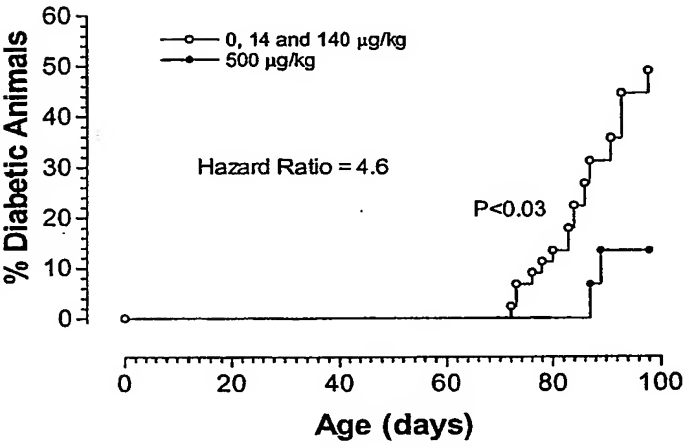


Figure 4.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 July 2003 (17.07.2003)

PCT

(10) International Publication Number
WO 2003/057204 A3

(51) International Patent Classification[7]: C07D 313/14, A61K 31/335, C07D 337/14, 405/06, C12N 9/00, 15/54, C12Q 1/48, 1/68, G01N 33/573

(21) International Application Number:
PCT/EP2003/000079

(22) International Filing Date: 7 January 2003 (07.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2002 00026	8 January 2002 (08.01.2002)	DK
60/346,709	8 January 2002 (08.01.2002)	US

(71) Applicant *(for all designated States except US)*: **NORDIC BIOSCIENCE A/S** [DK/DK]; Herlev Hovedgade 207, DK-2730 Herlev (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CLOOS, Paul, Andreas, Compare** [DK/DK]; Gl. Kalkbraenderivej 62, 5.tv., DK-2100 Kobenhavn 0 (DK).

(74) Agent: **SMART, Peter, J.**; W.H. Beck, Greener & Co., 7 Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
5 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/057204 A3

(54) Title: MODULATION OF IAMT (PIMT OR PCMT) IN IMMUNE SYSTEM

(57) Abstract: Modulation of L-Isoaspartyl (D-Aspartyl) O-Methyltransferase activity in immune system associated cells enables the prevention or alleviation of an autoimmune response by decreasing self-antigen presentation or T-cell proliferation.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D313/14 A61K31/335 C07D337/14 C07D405/06 C12N9/00
C12N15/54 C12Q1/48 C12Q1/68 G01N33/573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C12N C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, SEQUENCE SEARCH, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 726 265 A (CIBA GEIGY AG) 14 August 1996 (1996-08-14) formula 1 ---	1-8
X	US 5 780 500 A (BETSCHART C ET AL) 14 July 1998 (1998-07-14) formula 1 ---	1-8
X	GB 1 080 979 A (J U GEIGY A G) 31 August 1967 (1967-08-31) formula 1 ---	1-8
X	US 3 641 056 A (SCHINDLER W ET AL) 8 February 1972 (1972-02-08) column 1, line 55 - line 62 --- -/--	1-8

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 July 2003	24. 09. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernando Farieta

INTERNATIONAL SEARCH REPORT

In... nal Application No
PCT/EP 03/00079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 45422 A (ZIMMERMANN KASPAR ;CIBA GEIGY AG (CH); ROGGO SILVIO (CH); BETSCHAR) 4 December 1997 (1997-12-04) claims 1-23	1-8
X	US 3 100 207 A (ZIRKLE CHARLES L) 6 August 1963 (1963-08-06) column 1, line 16	1-8
X	WO 98 15647 A (CIBA GEIGY AG ;FUERST PETER (CH); HUEBSCHER KAREN (CH); ROVELLI GI) 16 April 1998 (1998-04-16) claims 1-15	9-18
X	WO 01 83719 A (MEYERS RACHEL A ;WILLIAMSON MARK (US); MILLENNIUM PHARM INC (US)) 8 November 2001 (2001-11-08) claim 36	9-18
X	WO 02 052016 A (BAYER AG ;ZHU ZHIMIN (US)) 4 July 2002 (2002-07-04) claims 1-71	9-18
X	PERNA A F ET AL: "Metabolic consequences of Hyperhomocysteinemia in uremia." AMERICAN JOURNAL OF KIDNEY DISEASES, vol. 38, no. 4 suppl 1 , October 2001 (2001-10), pages s85-s90, XP002902941 ISSN: 0272-6386 the whole document	9-18
A	GB 1 098 347 A (GEIGY AG J R) 10 January 1968 (1968-01-10) claims 1-13	1-8
A	ZIMMERMANN K ET AL: "Dibenzoxepines as treatments for neurodegenerative diseases." PURE APPL. CHEM., vol. 71, no. 11, 1999, pages 2039-2046, XP002902886 the whole document	1-8
A	DOROTEA MÜCK-SELER ET AL: "TCH-346 novartis." I DRUGS, vol. 3, no. 5, 2000, pages 530-535, XP002902887 ISSN: 1369-7056 RN 181296-84-4.	1-8

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/00079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 02 08395 A (MEYERS RACHEL ;TSAI FONG YING (US); MILLENNIUM PHARM INC (US)) 31 January 2002 (2002-01-31) claim 22	9-18
A	TSAI W ET AL : "Amino acid polymorphisms of the human L-isoaspartyl/D-asparty1 methyltransferase involved in protein repair." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 203, no. 1, 30 August 1994 (1994-08-30), pages 491-497, XP002902942 ISSN: 0006-291x table 1	17
A	HUEBSCHER K J ET AL : "Protein isoaspartyl methyltransferase protects from Bax-induced apoptosis." GENE, vol. 240, 1999, pages 333-341, XP002902939 ISSN: 0378-1119 the whole document	9-18
A	XIAODONG CHENG ET AL : "Adomet- dependent methylation, DNA methyltransferases and base flipping." NUCLEIC ACIDS RESEARCH SURVEY AND SUMMARY, vol. 29, no. 18, 2001, XP002902940 figure 3	9-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/00079

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7 10-18
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 1, 9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8

   Claims 1-8 directed to derivatives of 10-aminoaliphatyl-dibenz[b,f]oxepine for the preparation of compositions for the prevention, alleviation or treatment of an autoimmune response and/or disease in a mammal.

2. Claims: 9-18

   Claims 9-18 directed to IAMT encoding sequences or derivatives for the preparation of compositions for the prevention, alleviation or treatment of an autoimmune response and/or disease in a mammal.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 7 10-18

Claims 7 and 10-18 relate to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practised on the human or animal body (Rule 39.1 (iv)). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds /compositions.

Continuation of Box I.2

Claims Nos.: 1, 9

The wording " regulator " in claim 1 and " IAMT or a functional derivative " in claim 9 reveal a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breaadth of the claims is impossible.

Consequently, the search has been restricted to :
Claim 1: the compounds comprise by formula I and the specific compounds named in claims 5 and 6.
Claim 9: the known nucleic sequence of IAMT or PIMT or PCMT related to diseases.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.